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EVIDENCE OF MIGRATION OF INGESTED ASBESTOS INTO VARIOUS BABOON ORGANS

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Abstract

This report presents the results of transmission electron microscopic analysis for asbestos in ten organs of the newborn baboon who was orally administered with chrysotile asbestos. The feeding period consisted of 9 days with a cumulative dose of 3×10^{13} chrysotile fibers per kilogram. Analysis of the same ten organs of a control newborn baboon is also included.

The test tissues showed higher levels of asbestos than respective control tissues. The highest concentration of chrysotile fibers was found in the kidney cortex followed by lymph nodes, spleen, colon, esophagus, kidney medulla, stomach, and liver. Fiber size distribution of the fibers recovered indicated that the longest fiber was retained in the kidney cortex.

KEY WORDS: Low temperature ashing; Specimen preparation; Microliter drop technique; Asbestos in tissues; Migration of asbestos; Oxygen plasma; Chrysotile asbestos; Asbestos ingestion; Asbestos fiber size; Asbestos feeding studies.

Introduction

Exposure to asbestos occurs to small numbers of people in asbestos industry or to the general population through air, food, water, and beverages.[1-6] A few references are cited here from the extensive literature on lung cancer, mesothelioma, and asbestosis occurring from exposure to occupationally inhaled asbestos.[7-10] Hazards of orally ingested asbestos are not yet understood. Reports have appeared showing that the ingested fibers penetrate, migrate between, and are recoverable from the gastrointestinal tract and distant tissues,[11-13] as well as from urine.[14-15]

Chrysotile asbestos fiber counts in drinking water have been up to 36 million fibers/ℓ (0.36µg/ℓ) in Bay Area Counties and 143 million fibers/ℓ (0.3µg/ℓ) in Everett, Washington.[3] An understanding of how the body handles ingested asbestos is important in determining the potential health effects of asbestos in drinking water, foods, drugs, and beverages. Little information is available on the quantitative levels of fibers retained by various organs of the body following the oral exposure to asbestos. A newborn baboon fed on asbestos-containing milk formula represents an ideal simulation of a newborn human baby feeding on bottle milk prepared from milk concentrate and drinking water, 1:1 by volume. This paper presents the results of a study conducted to determine if orally administered asbestos in a non-human primate baboon, can be recovered from the gastrointestinal tract and from organs other than the gastrointestinal tract, and in what concentrations and fiber size distribution.

Materials and Methods

Two neonate baboons *(Papio anubis)*, born exactly 8 days apart, were used as control and test animals. Each animal was maintained in an Isolette infant incubator under conditions of controlled temperature, humidity and air supply. The test animal weighed 920 grams and was bottle fed with a suspension of UICC Canadian chrysotile in milk formula. See Table 6 for the frequency of distribution of UICC fiber lengths. The suspension was prepared by swirling 10 mg of asbestos in 500 ml of distilled water which had been previously filtered through a 0.1 µm Millipore membrane. It was mixed 1:1 with concentrated milk

formula (Similac, Ross Laboratories) prior to feeding to give 10 mg of chrysotile per liter. Assuming 10^6 fibers per nanogram, the feeding suspension contained 10^{13} fibers per liter (F/ℓ). A total of 2.8 liters was provided *ad libitum* to the test animal weighing 920 g over a period of 9 days beginning 24 hours after birth. Therefore, the total dose for the test animal was approximately 3.0×10^{13} F/kg. The control animal weighed 961 g and was bottle fed with a one to one mixture of concentrated milk formula and distilled, filtered water; 2.6 liters were consumed over 9 days. Both animals were sacrificed at the end of the 10-day trial.

Tissue Preparation

This paper describes a method developed for retrieval of asbestos from animal tissues for counting and sizing it under transmission electron microscope (TEM). Scanning electron microscopy was not used due to difficulty in identifying small fibrils.

1. Comparable weights of control and test tissues were dried in a vacuum oven at 90°C until weights became constant.
2. Dried tissue was ashed in a low temperature plasma asher at 80 watts for about 3 hours.
3. The resultant ash was suspended in 10 ml of 1% acetic acid and ultrasonicated at 50kHz for 1 minute to obtain fiber dispersion without breakage. Treatment with acid helped remove inorganics and seemed to improve removal of organics in the subsequent ashing step. A check using reference UICC Canadian chrysotile indicated that acid treatment did not disturb the morphology or diffraction pattern under transmission electron microscope.
4. The suspension was filtered through a 0.1 μm Nuclepore membrane and the latter was ashed in the plasma unit at 80 watts for 3 hours. The residue was suspended in 5 ml of filtered (0.2 μm Fluoropore) acetone and sonicated for one minute.
5. The volume of acetone-tissue ash suspension to be deposited on Formvar and carbon-coated TEM grid depended upon the dry weight of each tissue sample. Rapid drying of the suspension delivered from a microliter syringe was effected by the use of an infrared lamp (250 W) at four-five inches from the grid. Acetone suspensions were kept covered and cold to minimize evaporation of acetone during grid preparation.
6. Between samples, the syringe was rinsed several times with filtered acetone. Syringe contamination was not a problem as evidenced by the below-detection results obtained for several of the tissue preparations. It is important to note that test and control tissues were prepared in random sequence.

Using the above procedure, TEM specimens of various organs were prepared with practically no difference in preparative difficulties.

Electron Microscopic Scanning and Calculations

The grids were examined at a magnification of 19,000 under TEM Philips 300. Morphology and occasionally selected area electron diffraction (SAED) were used to identify chrysotile asbestos. Fifty grid holes, 25 from each of two grids prepared from same ash suspension, were scanned for each control and test tissue. Fibers recovered in control samples served not only as count of the control tissues, but also as background count of all the materials and procedures used in preparing the grids.

Fibers were counted and sized for length and width. In case of a bundle or cluster, the length of the longest fiber and the width of the widest region were measured. A bundle or cluster was counted as one fiber. Note that a bundle is defined here as two or more fibrils touching each other along their long axes, and a cluster is that which is composed of two or more individual fibers and/or bundles oriented at various angles to each other, except parallel at any point of contact. The fibers were identified and graded[15] as Class A, Class B, and Class C as follows:

Class A - Fibers which were clearly representative of chrysotile morphology and sometimes SAED.

Class B - Fibers which were somewhat degraded, but still having the morphology of chrysotile.

Class C - Fibers which were degraded and barely identifiable by morphology.

The final concentration of fibers per mg dry weight of tissue was calculated by employing the total of fibers in Class A and Class B recovered in 50 grid holes per tissue. The equation used for calculations was as follows:

Fibers/mg dry weight of tissue =

$$\frac{F \times A_1 \times V_1}{G \times A_2 \times V_2 \times M} \quad \text{.....(i)}$$

$$\frac{F \times 80}{V_2 \times M} \quad \text{.....(ii)}$$

where F = total number of fibers recovered per tissue

A_1 = 0.07 cm^2 = area of a 200 mesh TEM

V_1 = 5 ml = volume of ash-acetone grid suspension

G = 50 = total number of grid holes examined per tissue

A_2 = 8 X 10^{-5} cm^2 = area of 1 grid hole

V_2 = μℓ volume deposited on grid

M = weight of dry tissue in mg

A loading factor, L, was derived to permit quick comparisons of fiber retention (1) between control and test tissues of the same type and (2) among different types of tissues within the test group, without conversion of the EM scanning data to a unit of fiber concentration/mg dry wt. of tissue. L equalled $V_2 \times M$. By substituting L for $V_2 \times M$ in the above equation, it can be rewritten as

$$\frac{F \times 80}{L} \quad \text{.....(iii)}$$

The value of L was maintained almost equal in the test and control pairs of tissues, as well as among the tissues by adjusting the volumes of suspensions to be deposited on grids. This was, in other words, to normalize the small variations in dry weights of tissue samples. As shown in Table 1, the values of L for 20 tissue preparations (excluding kidney cortex preparations since they were from a separate study) ranged from 0.89 to 1.086. Since the volume of total

suspension, V_1, was five in each case, the actual dry weight per TEM grid surface ranged within 0.18 to 0.22 mg for 20 tissues.

Detection Limit

The detection limit of this analytical technique can be calculated by substituting values of F and L in the above equation (iii). If one fiber was found per 50 grid holes scanned and if L was one, the detection limit would be 80 F/mg dry weight. The detection limit can be improved by viewing more grid holes or by increasing V_2 and/or M, which in turn, may be limited by the amount of extraneous material on the grid surface to obstruct in the scanning under the TEM. The above detection limit is almost twice as sensitive as that obtained by Cunningham, et al.[16]

Results and Discussion

Tissue preparative technique reported here for TEM analysis of asbestos fibers has the following merits:

1. It can be used for analysis of fresh, frozen or formalin preserved tissues, and fresh filtered urine.
2. The sample suspension can be preserved by allowing the acetone to evaporate under cover and freezing it. Since leaching of asbestos metals is prevented during such preservation in dry state, fresh grids can be prepared for future TEM analysis by resuspending the ash in acetone.
3. Since almost equal weights of the different tissues under analysis are deposited on each grid surface [by adjusting the dry weight of tissue times the volume of suspension on a grid (total volume of suspension)], the fiber count itself permits instant comparisons of the levels of fibers in the different tissues.
4. The detection limit is very good.

Two grids each prepared from the test and control kidney cortex tissues were coded and sent to another laboratory. On uncoding, the results on the fiber count were found to be remarkably close to those obtained at this laboratory indicating the reliability of this analytical technique. This technique differs from that used by Cunningham, et al.[16-17] in two respects: tissue ash was solubilized in acetic acid instead of hydrochloric acid; and, the ash of the filter bearing the tissue ash was suspended in acetone instead of water for the purpose of transferring it onto a TEM grid.

Using the above technique grids were prepared for TEM analysis of eleven tissues each, of control and test neonate baboons. Chrysotile asbestos count and size data were obtained by examining 50 grid holes, 25 each on two grid preparations from each tissue.* Table 1 shows the results on the recovery of asbestos in eleven tissues of a test baboon on a gradient of high to low contents. In general, the number of fibers recovered in the tissues of control animal was low. Concentrations of fibers (F/mg dry weight) in the test tissues were calculated after correction for the fibers found in the respective tissues of the control neonate baboon. Retention of fibers in test cecum could not be evaluated because more fibers were recovered in control sample than in the test sample.

Each pair of data from test and control samples was compared using t-test,[18] and $P \leq 0.005$ was interpreted as indicating significant difference. A highly significant difference ($P \leq 0.001$) was found between the paired means of fibers recovered in kidney cortex and in lymph nodes.

In our experimental situation, the sources of variation contributing to the variance were treatments, tissues and grids. Tissues from control and treated animals were considered as samples and the grids as subsamples.

Nested analysis of variance or analysis of variance with subsamples was performed to test the particulate distribution on grids prepared from the same sample. A significant difference ($P=0.005$) was found between two populations (grids) when the fiber counts derived from all tissues were compared. Hence, comparisons were made using Welch's t-test between the fiber counts recovered in 25 grid holes of two grids each for each of the ten tissues. Eight of the ten tissues did not show significant difference ($P=0.005$) between the replicate counts of two grids prepared from each. Fiber counts were significantly different between two grids prepared from each kidney cortex and spleen ($P=0.005$).

Concentrations of chrysotile fibers recovered in different test tissues (corrected for control values) were statistically evaluated for multiple comparisons. Table 2 shows P values for comparison between the fiber levels of any two tissues; for example, when kidney cortex was compared individually with seven other tissues it showed statistically significant excess of asbestos retention ($P \leq 0.001$). A similar high P value was obtained when comparisons were made between the fiber levels in lymph nodes and kidney medulla, lymph nodes and stomach, lymph nodes and liver, and colon and liver. Differences were significant at $P=0.005$ between the fiber levels in spleen and stomach, spleen and liver, and colon and stomach. Caution is required in drawing inferences from the results wherever the fiber counts are very low (See Table 1).

Tables 3-5 show fiber size of chrysotile fibers recovered in test tissues. The total number of fibers recovered in each tissue is included in these Tables because the generated data are influenced by the total number. The range in length of fibers was 0.16 μm to 35.0 μm among all the tissues, and was 0.16 μm to 9.30 μm among five gastrointestinal tract organs. The longest fiber was recovered from test kidney cortex. The diameters ranged from 0.01 μm to 1.50 μm among all the tissues and 0.02 μm to 0.16 μm among five gastrointestinal tract organs. Wide ranges of length, diameter, aspect ratio, and recovery of bundles and clusters in all organs (including colon wherein several bundles of degraded morphology Class C, were recovered) suggest that the processes of penetration and migration occur for all sizes. It should be noted that above information relating to size was

* Sixty-two and 60 grid holes, respectively, were examined on the grids prepared from test and control kidney cortex tissues.

Table 1. Chrysotile asbestos recovered in tissues of neonate baboons

	Tissue weight (mg) per grid		Loading factor, L		Fibers recovered 50 grid holes, F*		Concentration fibers/mg dry wt.**
	Test	Control	Test	Control	Test	Control	Test
Kidney cortex†	0.19	0.16	0.948	0.798	109	0[a]	7789
Lymph nodes	0.21	0.20	1.074	1.066	24	5[a]	1415
Spleen	0.20	0.20	1.010	1.000	15	2[b]	1030
Colon	0.20	0.20	0.991	1.019	12	2[b]	807
Esophagus	0.20	0.21	1.013	1.046	8	1[c]	553
Kidney medulla	0.19	0.18	0.940	0.910	4	0[c]	340
Stomach	0.20	0.21	0.977	1.051	2	1	82
Liver	0.20	0.18	0.980	0.890	1	0	82
Duodenum	0.20	0.32	0.996	1.086	3	3	-
Cecum	0.20	0.20	1.023	0.994	5	8	-
Heart	0.20	0.20	1.002	1.023	BDL††	0	BDL††

* Preparations from test and control kidney cortex were examined for 62 and 60 grid holes, respectively.

** Fibers/mg dry wt. = $\frac{F \times 60}{L}$. The values shown were after correction for control levels.

† Kidney cortex data was taken from Patel-Mandlik, et al.[13] †† Below Detection Limit

[a]$P \leq 0.001$, [b]$P=0.01$, [c]$P=0.05$ are for comparison between test and control tissues

Table 2. Statistical difference* in the fiber levels retained by baboon tissues - Comparisons between each two tissues

Tissue	No. of Fibers	Kidney Cortex	Lymph Nodes	Spleen	Colon	Esophagus	Kidney Medulla	Stomach	Liver
Kidney Cortex	109		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lymph Nodes	24			0.25	0.1	0.01	0.001	<0.001	<0.001
Spleen	15				>0.75	0.25	0.25	0.005	<0.005
Colon	12					0.5	0.1	0.005	<0.001
Esophagus	8						0.5	<0.1	<0.01
Kidney Medulla	4							<0.25	0.1
Stomach	2								0.1
Liver	1								

*Based on number of fibers shown in column 2

Table 3. Length data in μm of chrysotile fibers recovered in test baboon tissues.

Tissue	Mean	Standard deviation	Coefficient of variation	Maximum	Minimum	Median	Geometric mean
Kidney cortex	1.7(109)*	4.38	257	35	0.24	0.50	0.73
Lymph nodes	0.86(24)	0.83	97	3.30	0.28	0.56	0.65
Spleen	1.87(15)	3.29	174	12.80	0.20	0.60	0.87
Colon	0.33(12)	0.22	64	0.80	0.16	0.14	0.29
Esophagus	1.16(8)	1.04	90	2.84	0.22	0.70	0.76
Kidney medulla	0.42(4)	0.10	22	0.50	0.30	0.50	0.42
Stomach	0.18(2)	0.06	16	0.40	0.32	-	0.36
Liver	4.80(1)	0.00	0	4.80	4.80	-	4.80
Duodenum	4.20(3)	4.54	108	9.30	0.60	2.70	2.47
Cecum	0.52(5)	0.50	94	1.40	0.24	0.28	0.40

* Figures in parentheses were total number of fibers recovered

Table 4. Fiber diameter data in um of chrysotile asbestos recovered in test baboon tissues.

Tissue	Mean	Standard deviation	Coefficient of variation	Maximum	Minimum	Median	Geometric mean
Kidney cortex	0.04(109)*	0.04	100	0.34	0.03	0.03	0.04
Lymph nodes	0.08(24)	0.08	107	0.32	0.01	0.04	0.05
Spleen	0.15(15)	0.37	241	1.50	0.02	0.04	0.06
Colon	0.05(12)	0.04	74	0.14	0.02	0.04	0.04
Esophagus	0.07(8)	0.02	31	0.11	0.03	0.08	0.07
Kidney medulla	0.05(4)	0.02	40	0.08	0.04	0.04	0.05
Stomach	0.08(2)	0.06	71	0.12	0.04	-	0.07
Liver	0.12(1)	0.00	0	0.12	0.12	-	0.12
Duodenum	0.08(3)	0.074	96	0.16	0.02	0.05	0.05
Cecum	0.06(5)	0.02	34	0.08	0.04	0.08	0.06

*Figures in parentheses were total number of fibers recovered

TABLE 5. TYPE AND SIZE OF FIBERS RECOVERED IN TEST BABOON TISSUES

Tissue		Kidney Cortex	Lymph Nodes	Spleen	Colon	Esophagus	Kidney Medulla	Stomach	Liver	Duodenum	Cecum
Recovery of fibers	Classes* A+B	109	24	15	12	8	4	2	1	3	5
	Class** C	0	2	0	21	0	0	0	0	1	1
	Per Cent Bundles/ Clusters	Not recorded	29	33	0	37.5	25	50	100	66	20
Aspect Ratio	Mean	39.50	22.58	19.91	7.85	15.57	8.95	6.33	40.0	108.25	7.85
	Max.	800.0	104.0	70.0	12.0	35.50	12.50	10.0	40.0	186.0	7.50
	Min.	5.0	1.75	7.5	3.75	3.60	6.25	3.67	40.0	3.75	3.25

*Fibers graded as Classes A and B were included to obtain the fiber concentration in the tissues.
**Fibers of Class C indicated the degradation.

Table 6. Percent frequency of fiber lengths of UICC standard Canadian chrysotile and chrysotile fibers recovered in test baboon tissues

Length in um	UICC*	Kidney cortex	Lymph nodes	Spleen	Colon	Esophagus	Kidney medulla	Stomach	Liver	Duodenum	Cecum	Heart
0.2-0.5	38.6	37.6	37.5	40.0	83.3	50	100	100			80	
0.5-1.0	33.4	26.6	37.5	26.7	16.7	12.5				33.3		
1-2	19.8	23.0	12.5	13.3		1.5					20	
2-5	13.2	5.5	12.5	6.7		25			100	33.3		
5-10	1.76	1.7		6.7						33.3		
10-25	0.93	2.7		6.7								
25-50	0.24	0.9										
50-100	0.07											
Total No. of fibers recovered*		109	24	15	12	8	4	2	1	3	5	BDL‡

* Data from Rendall, R.E.G.[19]
• Per cent frequency data should be considered in the light of total number of fibers recovered.
‡ Below detection limit

based on a relatively small number of fibers recovered from various organs (See Tables 3 and 4). A cluster of fibers recovered in test spleen is illustrated in Figure 1.

Fig. 1 A cluster of chrysotile asbestos fibers recovered in the spleen of test neonate baboon. Bar length = 1.0 µm.

Frequency of distribution of fiber lengths (Table 6) of retained fibers in kidney cortex, lymph nodes and spleen shows a general pattern almost comparable to the distribution frequency of administered fibers.[19] Relatively, low retention of fibers in the remaining tissues does not permit definitive comparisons of frequency of size distribution among different organs.

It is hoped that the information provided in this paper when combined with epidemiological data on cancer deaths would contribute towards fuller understanding of carcinogenicity of asbestos.

Acknowledgements

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Discussion with Reviewers

A.M. Langer: Did you monitor the "ambient" environment for the presence of asbestos fibers?

ASBESTOS IN BABOON ORGANS FOLLOWING ITS INGESTION

Authors: The control newborn baboon was housed in a chamber with controlled temperature, humidity, and filtered air (0.1 um) immediately after birth. There was no asbestos insulation in the walls of baboon room. The newborn was fed only milk formula prepared in filtered water (0.1 um) for nine days and was sacrificed on the tenth day.

J.T. Stasny: What was a typical total dry weight for kidney tissue used, prior to digesting and subsquent sampling for asbestos?
Authors: The dry weights of control and test kidney cortex were 133mg and 158mg respectively.

P.P. McGrath: What was the final volume (V_2) of acetone suspension per grid?
Authors: V_2 ranged from 3 to 33 µl, depending on the weight of the tissue sample.

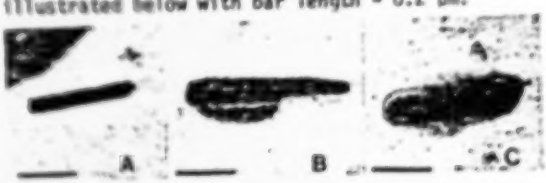
J.T. Stasny: Was obscuration of asbestos with nonasbestiform inorganic residues a problem during your observations?
Authors: The preparations were pretty clean. There were hardly any residues interfering with TEM analysis of asbestos fibers. This could be due to the fact that the animals were fed formula only.

A.M. Langer: Is there any fiber loss observed when the tissue specimens are ashed in a low temperature plasma?
Authors: It is quite possible that some fibers are lost in the low temperature plasma asher even though extreme care is taken in using tall ashing beakers, low oxygen flow rates, etc. One of us, J. M., has estimated loss of fibers on ashing moderately loaded fibers to be less than 1%.

W.P. Cunningham: Why did you filter and ash the samples twice? How do you know that this treatment doesn't affect the observed fiber number in your samples? Have you compared your technique to other methods of preparation such as that described by Cook and Olson, Science 204, 195-198, 1979; or Stasny et al., Scanning Electron Microscopy/1979/I, 587-595.
Did you do any control experiments in which a known concentration of fibers were carried through your procedure for ashing and dispersion of fibers to determine if fibers are lost, damaged or released from bundles or clusters by your techniques?
Authors: The samples examined were pieces of tissue. The first ashing broke down the tissue. The second ashing further eliminated the organic constituents of the tissue, and was essential for rendering preparation appropriate for examination under the TEM. There was only one filtration in our preparation procedure. The ultrasonic bath was kept at a low level to prevent breakage of long fibers into a number of shorter fibers. No known "standard sample of asbestos" in tissue or even in water suspension is available to fully test the possibility of fiber loss. Tests with fibers on filters indicate that fiber loss is less than 1%. The method described by Cook and Olson (1979) is for liquid urine and not directly applicable to samples of tissue. Both the techniques described in Stasny et al. (1979) and in Cook's "Preparation of extrapulmonary tissues and body fluids for quantitative transmission electron microscope analysis of asbestos and other mineral particle concentrations (Annals NY Acad. Sci. 330, 717-724, 1979) are being studied and some comparisons of techniques will be made.

A.M. Langer: The authors describe "Class C fibers" as degraded. What is the form of this "degradation", what magnifications are required to see these characteristics(are magnifications in excess of the scan magnification required to see these alterations), and what proportion of this degradation may be attributed to beam damage? Does the extraction treatment and physical manipulation have a role in the alteration of fiber morphology?
Authors: Chrysotile fibers were identified as Class C fibers when morphological appearance was that of chrysotile except that edges and central canal were not distinctly seen throughout the length of the fiber (Figure C, below). Magnification in excess of the scan magnification (19,000x) was not required to see these alterations. It is unlikely that beam damage was responsible for fiber degradation because when examined at magnification of 19,000x,degradation, if present, was noticed at the first sight so that fibers could be placed in Class A, B or C without prolonged exposure.

Degraded fibers were not recovered in the preparation of Standard chrysotile fibers subjected to our extraction treatment and other procedural steps. Fibers of A, B and C classes are illustrated below with bar length = 0.2 um.



Above fibers were recovered from urine specimens of juvenile baboon gavaged with chrysotile asbestos (separate study), but are shown here to illustrate the degrees of degradation which were encountered in the present study.

W.P. Cunningham: What is your operational definition of a fiber in terms of aspect ratio? How do class C fibers differ from rectangular silicatons crystals, plant materials, etc.?
Authors: A fiber is defined as an elongated particle having a length to width ratio of at least 3:1. Class C fibers were identified by their smooth walls and somewhat visible central canal (see Figure C above). The fibers could not have been from feed since the test animal was newborn, was maintained in an environment kept strictly free from asbestos and, was fed only milk formula prepared in filtered water. Class C fibers were not included in the statistical analysis.

A.M. Langer: The investigators classify fibers on the basis of morphology and structural characteristics. The fiber counts represented in their tables do not reflect these differences. Could the authors indicate why these

characteristics were omitted from their tables or inform the reader as to their relative occurrence in each of the specimens analyzed?
Authors: The fibers recovered from tissues of test baboon are reported as Class A+B fibers and Class C fibers in Table 5. Majority of the fibers listed in A+B group (Table 5) belonged to class A fibers, as opposed to approximately equal numbers of class A and class B fibers recovered from urine of a gavaged juvenile baboon (text reference 15).

P.P. McGrath: Did you try SAED on any of the class B and C fibers?
Authors: Yes. Class B and C fibers did not diffract.

J.T. Stasny: The use of SEM was summarily dismissed as a method for identifying small fibrils, yet the fiber dimensions presented are within the resolution limits of modern research SEM's. Enhancement of image contrast of the fibrils for SEM detection will increase fibril detectability, as well as the utility and the value of the techniques you report, by making the techniques more widely applicable. Would you please comment.
Authors: There is no reason why the preparation techniques described here would not be applicable to SEM examinations. One of us, J. M., has imaged small fibers but found identifying small chrysotile fibrils in the SEM difficult. The limits of precision of fiber counts by EDXA in the SEM are shown by Chatfield and Dillon (Scanning Electron Microsc. /1978/I, 487-495). A combination of the TEM with EDXA and SAED represents the most complete analytical facility available. Methods for enhancement of image contrast of the fibrils are currently being tested with some moderate success. The authors found the central canal in chrysotile which is observed in the TEM very helpful in identifying the mineral fibers.

W.P. Cunningham: Would you have counted all the fibers shown in the cluster in figure 1 as a single fiber? How do you distinguish between fibers that were really attached to each other and those that happened to be deposited in a cluster during drying?
J.T. Stasny: It seems that the counting of fibers would be difficult, judging from the type of fiber aggregation shown in figure 1. Were aggregates of this type "normal" for all of the dispersed samples examined?
Authors: In our analysis, any size bundle or cluster was counted as one fiber. The problem of counting clumps is most difficult and no consencous among analysts exists. Some fiber clumps are very intertwined as if closely attached. Some clustering of fibers during filtration may occur. Since acetone evaporates rapidly, especially under infrared lamp, cluster formation during drying is not likely. Random sampling of 25 grid holes of two grids each prepared from the same acetone suspension showed no statistically significant difference in the fiber counts.

W.P. Cunningham: What was the frequency of clusters of the type shown in figure 1 in your various samples. Could these large, heterogenous clusters be eliminated or reduced by preparing the sample over and reducing the loading factors?
Authors: Reducing the loading factor might reduce large clusters if they are not originally in the tissue but this would also reduce the detection limit of the analysis. Figure 1 illustrates a large cluster, the only one of its type recovered in our tissue samples.

A.M. Langer: Would the authors like to comment on the difficulties involved in the conversion of fiber counts to fiber mass?
Authors: No acceptable procedure exists for determining the mass of asbestos in a water sample directly. A mass value for a sample is computed using data on the fiber concentration, the lengths and widths of the fibers observed, and the density of the asbestos type involved.

It was difficult to measure dimensions of a large size cluster or bundle of fibrils. Hence, length of the longest fibril and width of the widest region of fiber aggregation were measured. Values of mass in $\mu g/g$ dry wt. $\times 10^{-3}$ in various organs follows:

Kidney cortex	42.409	Kidney medulla	0.008
Lymph nodes	0.264	Stomach	0.005
Spleen	1.258	Liver	0.138
Colon	0.020	Duodenum	0.161
Esophagus	0.091	Cecum	0.018

J.T. Stasny: What is the significance of your observation that distribution frequency of lengths recovered in kidney cortex, lymph nodes and spleen showed a general pattern almost comparable to distribution frequency of administered fibers?
Authors: Above observation suggests that our preparative procedures may not be causing detectable changes in the sizes of administered fibers. From recently published data (text reference 15 and, Cook and Olson, Science 204, 195-198, 1979) it appears that a selection process and/or breakage of fibers may occur prior to excretion of fibers in the urine. Frequency of occurrence of short fibers was greater in postgavage urine samples than in the material fed to the juvenile baboon (text reference 15). Fiber levels found in the urines of people who drank unfiltered Duluth water were somewhat shorter than those found in Duluth water samples (Cook and Olson, 1979).

M.L. Mace: What are the fiber concentrations found in blood, urine and feces? To what extent are fibers cleared through these routes after cessation of oral administration?
Authors: Blood, urine and feces were not examined in this particular baboon study. However, such work with other baboons is currently underway sponsored by an EPA Grant at the University of Illinois. Some preliminary results can be found in text reference 15.

M.L. Mace: Do the organs which contain higher number of fibers in these acute exposures also have a higher incidence of asbestos-related diseases after long-term exposure?
Authors: No long-term exposure studies of baboons have been conducted.